

APPLICATION OF THE FISH GUT ASSOCIATED
SELECTED MICROBES IN FISH FEED USEFUL
IN ENHANCING THE GROWTH PERFORMANCE
OF THE JUVENILE TROPICAL MALAYSIAN
BROWN MARBLED GROUPEL,
Epinephelus fuscoguttatus

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DOCTOR OF PHILOSOPHY
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Application of the fish associated selected microbes in fish feed
useful in enhancing the growth performance of the juvenile
tropical Malaysian brown marbled grouper, epinephelus
fuscoguttatus / Mithun Sukumaran.

PERPUSTAKAAN SULTANAH NUR ZAHIRAH
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OF THE JUVENILE TROPICAL MALAYSIAN
BROWN MARBLED GROUPER,
*Epinephelus fuscoguttatus***

MITHUN SUKUMARAN

**Thesis submitted in Fulfillment of the Requirement
For the Degree of Doctor of Philosophy in the
Institute of Tropical Aquaculture
University Malaysia Terengganu**

June 2010

Dedicated to all the well wishers.....

Abstract of thesis presented to the Senate of University Malaysia
Terengganu in fulfillment of the requirement for the
Degree of Doctor of Philosophy

**APPLICATION OF THE FISH GUT ASSOCIATED SELECTED MICROBES
IN FISH FEED USEFUL IN ENHANCING THE GROWTH PERFORMANCE
OF THE JUVENILE TROPICAL MALAYSIAN BROWN MARBLED
GROUPEL, *Epinephelus fuscoguttatus***

MITHUN SUKUMARAN

June 2010

Chairman : Associate Professor Anuar bin Hassan, Ph.D.
Member : Professor Mohd. Effendy bin Abdul Wahid, Ph.D.
Dr. Anil Chatterji, Ph.D.

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Brown marbled grouper (BMG)- *Epinephelus fuscoguttatus* is one of the most important species among the cultured groupers in Malaysia, Indonesia and Thailand. The current research focused on the isolation of selected microbes associated with fish guts and incorporation of these microbes in fish feed to assess the enhancement of growth performance of juveniles of *E. fuscoguttatus*. Initially an attempt was made to optimize the culture conditions for better survival of the BMG juveniles. The oxygen consumption by the juveniles was considered as an indicator to assess the intensity of stress under different environmental conditions. The juveniles showed preference to temperatures lower than 30° C, slightly alkaline pH (8 pH) and 30 ppt (salinity) and as such these parameters were selected for conducting

preceding main experiments. The growth performance of juvenile of *E. fuscoguttatus* was also studied with the feeds incorporated with microbes isolated from the guts of the same species. The 11 gut isolates were identified using the 16S rDNA method as *Brevibacterium* sp., *Photobacterium damselae*, *Staphylococcus* sp., *Escherichia coli*, *Micrococcus* sp., *Pseudoalteromonas* sp., *Aspergillus* sp. and 2 species of *Ascomycota* sp. and fungal sp. For the confirmatory experiment, Feed A (control feed without any microbes), Feed B (*Brevibacterium* sp.), Feed C (*P. damselae*), Feed D (*Staphylococcus* sp.) and Feed E (mixture of *Brevibacterium* sp., *P. damselae* and *Staphylococcus* sp.) were prepared and their efficacy was studied. A microbial load of 10^5 - 10^6 cells/g of different microbes incorporated in feed was given for duration of 90 days. Maximum weight gain (11.42 ± 0.22 g) was recorded for Feed-E followed by Feed-D (11.09 ± 0.25 g), Feed-C (10.31 ± 0.23 g) and Feed-B (9.63 ± 0.62 g). However, the control Feed-A showed a minimum gain in weight (8.52 ± 0.21 g) during 90 day of experiment. A maximum average increase in weight (34.04%) was recorded with Feed-E after 90th day as compared to the Feed-A. Interestingly, relative growth rate, specific growth rate and food conversion ratio were better in feeds those were incorporated with microbes as compared to control feed. The theoretical weights during 90 days for Feed-A, Feed-B, Feed-C, Feed-D, and Feed-E were; $W_t = 33.5 [1 - e^{-2.48(t+0.001)}]$, $W_t = 48.9 [1 - e^{-2.54(t+0.001)}]$, $W_t = 46.3 [1 - e^{-5.80(t+0.003)}]$, $W_t = 61.9 [1 - e^{-2.57(t+0.002)}]$ and $W_t = 72.5 [1 - e^{-2.62(t+0.003)}]$ respectively calculated by applying von Bertalanffy's growth equation. *Brevibacterium* sp. showed the maximum growth at 28 ppt and at temperature of 30° C whereas *P. damselae* and *Staphylococcus* sp

28 ppt and 25° C and 20 ppt and 35° C respectively. All three feed with microbes showed viability at 4° C whereas microbes ($\geq 10^5$ cells) were found viable in diets till 30th day after the storage. The nutritional composition of microbes showed a strong evidence of their applicability in aquaculture. The protein contents were; 0.037, 0.132 and 0.05% for *Brevibacterium* sp., *P. damselae* and *Staphylococcus* sp.; respectively. As many as 18 types of fatty acids were identified from the three microbes. The presence of C15:0 (15.14 ± 3.64 mg g⁻¹) and C17:1 (13.89 ± 2.99 mg g⁻¹) in large quantities were observed in *Brevibacterium* sp. Interestingly, 17:1 (13.895 ± 2.99 mg g⁻¹) and 15:0 (15.14 ± 3.64 mg g⁻¹) with *P. damselae* while, 12:0 (0.645 ± 0.35 mg g⁻¹) was observed with *Staphylococcus* sp. Fatty acids such as 11:0 (undecanoic acid), 21:0 (heneicosanoic acid), 16:0 iso, 16:0 anteiso, 17:1 (heptadecenoic acid) and 18:3n6 (gammalinolenic acid) were present only in AQ 0719. 8:0 (octanoic acid), 10:0 (decanoic acid) and 12:0 (dodecanoic acid) were present in all the three microbes. Tetradecanoic acid (14:0) and 18:0 (octadecanoic acid) were present only in AQ 0731A. Heptadecanoic acid (17:0), 15:0 iso, 15:0 anteiso, 18:3n3 (alpha-linolenic acid) and 20:5n3 (eicosapentaenoic acid, EPA) were the fatty acids present commonly in *P. damselae*. High concentration of SAFA (Saturated fatty acid) was reported in *P. damselae* (56.92%). BRFA (Branched fatty acid) (10.59%) and MUFA (Monounsaturated fatty acid) (34.49%) were highest in *Brevibacterium* sp. while PUFA (Polyunsaturated fatty acid) (22.63%) and UNIFA (Unidentified fatty acid) were highest in *Staphylococcus* sp.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia
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**PENGGUNAAN MIKROB TERPILIH YANG BERKAITAN DENGAN USUS
IKAN YANG DIAPLIKASIKAN DALAM MAKANAN IKAN UNTUK
PENINGKATAN KADAR TUMBESARAN JUVENIL TROPICAL
MALAYSIAN BROWN MARBLED GROUPER,
*Epinephelus fuscoguttatus***

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June 2010

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Brown Marble Grouper (BMG) - *Epinephelus fuscoguttatus* merupakan salah satu spesies yang paling penting di antara spesies kerapu yang ditanak terutama di kawasan Asia termasuk Malaysia, Indonesia dan Thailand. Kajian terkini telah menumpukan kepada pemencilan mikrob daripada usus ikan dan diaplikasikan mikrob dalam makanan ikan bagi perkembangan tumbesaran juvenil *E. fuscoguttatus*. Dalam kajian ini, cubaan pengkulturan optimum juga telah dilakukan untuk mengkaji keupayaan tumbesaran juvenil BMG *E. fuscoguttatus*. Penggunaan oksigen oleh juvenil *Epinephelus fuscoguttatus* telah dilakukan sebagai pentunjuk kepada ukuran stres mengikut persekitaran yang berbeza. Keadaan yang sangat sesuai bagi juvenil dimana kadar penggunaan oksigen maksima telah diperhatikan pada

suhu di bawah 30° C, pH alkali (8 pH) 30 ppt (saliniti). Oleh demikian parameter ini dipilih sebagai petunjuk eksperimen utama. Bagi mengkaji tumbesaran dari segi penggunaan mikrob, mikrob yang didapati daripada usus spesies yang sama digabungkan dalam formulasi makanan. Mikrob yang dipencilkan dari 11 usus telah dikenalpasti dengan menggunakan kaedah DNA 16 S iatu ; *Brevibacterium* sp., *Photobacterium damsela*, *Staphylococcus* sp., *Escherichia coli*, *Micrococcus* sp., and *Pseudoalteromonas* sp., *Aspergillus* sp. dan 2 spesies *Ascomycota* sp. and Fungal sp. Kesahihan eksperimen Feed-A (makanan yang bersalut tanpa apa-apa probiotik) sebagai kawalan, Feed-B (*Brevibacterium* sp), Feed-C (*Photobacterium damsela*), Feed-D (*Staphylococcus* sp.) dan Feed-E (campuran *Brevibacterium* sp., *P. damsela* and *Staphylococcus* sp.) telah disediakan dan keberkesanannya telah diperhatikan. Kepadatan mikrob 10^5 – 10^6 cells/g bagi setiap mikrob yang diformulasi dalam makanan telah didedahkan untuk selama 90 hari. Pertumbuhan berat yang maksimum telah direkodkan untuk Feed- E ($11.42 \pm 0.22g$) diikuti dengan Feed-D ($11.09 \pm 0.25g$), Feed-C ($10.31 \pm 0.23g$) dan Feed-B ($9.63 \pm 0.62g$). Walaubagaimanapun kawalan (Feed-A) telah menunjukkan nilai minima 8.52 ± 0.21 g semasa eksperimen berlangsung selama 90 hari. Purata pertambahan berat (34.04%) telah direkodkan oleh kombinasi makanan (Feed-E) selepas hari ke -90 berbanding dengan makanan kawalan (Feed-A). Makanan ikan yang telah digabungkan dengan probiotik telah menunjukkan relative growth rate (RGR), specific growth rate (SGR), food conversion rate (FCR) yang lebih baik berbanding kawalan. Pertambahan berat secara teori selama 90 hari untuk Feed-A, Feed-B, Feed-C, Feed-D

dan Feed – E adalah masing-masing; $W_t=33.5 [1-e^{-2.48(t=0.001)}]$, $W_t=48.9 [1-e^{-2.54(t=0.001)}]$, $W_t=46.3 [1-e^{-5.80(t=0.003)}]$, $W_t=61.9[1-e^{-2.57(t=0.02)}]$ dan $W_t=72.5 [1-e^{-2.62(t=0.003)}]$ menggunakan persamaan Von Bertalanffy. *Brevibacterium* sp. telah menunjukkan tumbesaran maksima pada 28 ppt dan pada suhu 30° C dimana *P. damselae* dan *Staphylococcus* sp. masing-masing pada 28 ppt dan 25° C dan 20 ppt dan 35° C. Kadar hidup 3 mikrob dalam diet akuakultur didapati berupaya dalam diet untuk paras yang disyorkan iaitu pada suhu 4° C dan tahap ($\geq 10^5$ sel) hingga ke-30 hari tempoh simpanan. Komposisi nutrient mikrob telah menunjukkan bukti yang jelas kegunaan probion-probion dalam Akuakultur. Kandungan protein adalah 0.037, 0.132 dan 0.05 % masing-masing untuk *Brevibacterium* sp. *P. damselae* dan *Staphylococcus* sp. Sebanyak 18 jenis asid lemak telah dikenalpasti daripada ketiga-tiga mikrob tersebut. Kehadiran C15: O (15.14 ± 3.64 mg g⁻¹) dan C17:1 (13.89 ± 2.99 mg g⁻¹) dalam kuantiti yang banyak diperhatikan dalam *Brevibacterium* sp. Yang menariknya, 17:1 (13.895 ± 2.99 mg g⁻¹) dan 15:0 (15.14 ± 3.64 mg g⁻¹) pada *P. damselae*, manakala, 12:0 (0.0645 ± 0.35 mg g⁻¹) telah diperhatikan pada *Staphylococcus* sp. Asid lemak seperti 11:0 (asid undekanoik), 21:0 (asid heneeikosanoic) 16:0 iso, 16:0 anteiso, 17:1 (asid heptadecenoik) dan 18:3n6 (asid gammalinolenik) hanya terdapat pada *Brevibacterium* sp. Manakala 8:0 (asid oktanoik), 10:0 (asid dekanok) dan 12:0 (asid dodekanoik) telah didapati dalam kesemua ketiga-tiga probion. Asid tetradekanoik (14:0) dan 18:0 (Oktadekonoik) terdapat hanya dalam *P. damselae*. Heptadekanoik asid (17:0), 15:0 iso, 15:0 anteiso, 18:3n3 (asid alfa linolenik) dan 20:5n3 (asid eicosapentaenoik, EPA) adalah merupakan asid lemak yang hadir dalam *P. damselae*. Antara kesemua asid lemak,

SAFA adalah paling tinggi dalam *P. damselae* (56.92%). BRFA (Asid lemak bercabang) adalah (10.59%) dan MUFA (Asid lemak tak tepu mono) (24.49%) adalah paling tinggi dalam *Brevibacterium* sp. manakala PUFA (Asid lemak tak tepu pelbagai) (22.63%) dan UNIFA (Asid lemak yang tak dapat dikenalpasti) adalah paling tinggi dalam *Staphylococcus* sp.